Amendments to the Specification

Replace paragraph No. 111 on page 4 with the following paragraph:

--Figure 4 illustrates the λ-Red helper plasmid, pKD46 (Datsenko and Wanner, *supra;* ~~GenBank®~~ GENBANK Accession Number AY048746).--

Replace paragraph No. 124 on page 5 with the following paragraph:

--SEQ ID NO:1 is the first of two primer sequences used to PCR amplify the linear DNA fragment containing a kanamycin resistance gene from plasmid pKD4 (Datsenko and Wanner., *supra.*; ~~GenBank®~~ GENBANK Accession Number AY048743) designated as "T1(dxs)" and contains a homology arm designated as "h1" chosen to match sequences in the upstream region of the *ispA* stop codon (Figure 2).--

Replace paragraph No. 126 on page 5 with the following paragraph:

--SEQ ID NO:3 is the first of two primer sequence used to PCR amplify the linear DNA fragment containing a P_{T5} promoter comprising the –10 and –35 consensus sequences, lac operator (*lacO*), and ribosomal binding site (*rbs*) from plasmid pQE30 (~~Qiagen~~ QIAGEN Inc., Valencia, CA) and is designated as "T2(T5)" (Figure 2). The primer is later used to verify by PCR amplification the chromosomal construct of the integration of the kanamycin selectable marker and the P_{T5} promoter upstream of the *dxs* gene (Figure 6).--

Replace paragraph No. 127 on page 5 with the following paragraph:

-- SEQ ID NO:4 is the second of two primer sequences used to PCR amplify the linear DNA fragment containing a P_{T5} promoter comprising the –10 and –35 consensus sequences, lac operator (*lacO*), and ribosomal binding site (*rbs*) from pQE30 (~~Qiagen~~ QIAGEN, Inc.) and is designated as "B2(dxs)" and contains a homology arm designated as "h3" chosen to match sequences in the downstream region of the *dxs* start codon (Figure 2).--

Replace paragraph No. 157 on pages 5 and 6 with the following paragraph:

--SEQ ID NO:34 is the nucleotide sequence for plasmid pKD4 (Datsenko and Wanner, *supra*) having ~~GenBank®~~ GENBANK Accession number AY048743 and was used as a PCR template to amplify the DNA fragment containing a kanamycin resistance gene flanked by *FRT* site-specific recombinase recognition sequences.--

Replace paragraph No. 158 on page 6 with the following paragraph:

--SEQ ID NO:35 is the nucleotide sequence for plasmid pKD46 (Datsenko and Wanner, *supra*) having ~~GenBank®~~ GENBANK Accession number AY048746. Plasmid pKD46 expresses the components of the λ-Red Recombinase system.--

Replace paragraph No. 245 on page 13 with the following paragraph:

--The phage λ-Red recombinase system expressed on the helper plasmid pKD46 (~~GenBank®~~ GENBANK Accession number AY048746; SEQ ID NO:35; Figure 4) and

under control of an arabinose-inducible promoter was used, resulting in controllable and efficient *in vivo* triple homologous recombination. At least one of the two PCR-generated linear dsDNA fragments used during recombination was designed to contain a selective marker (kanamycin) flanked by site-specific recombinase sequences (*FRT*) (Example 1). The marker allowed for identification and selection of the cells that had undergone the desired recombination event. Once the constructs of the selected recombinants were verified by sequence analysis or PCR fragment analysis, the selective marker was excised by a second helper plasmid containing the site-specific recombinase gene under the control of the P_R promoter of λ phage (Examples 3 and 4). --

Replace paragraph No. 260 on page 15 with the following paragraph:

--The plasmids (pKD4, pKD46 and pCP20) used in the present invention have been previously described in the literature (Datsenko and Wanner, *supra*). The sequences for the λ-Red recombinase system components are contained on helper plasmid pDK46 (SEQ ID NO:35; ~~GenBank®~~ GENBANK Accession No. AY048746). The Flp/*FRT* site-specific recombinase helper plasmid used in the present invention was pCP20 (ATCC Number PTA-4455) Plasmid pKD4 (SEQ ID NO:34; ~~GenBank®~~ GENBANK Accession No. AY048743) was used as a template molecule for PCR amplification of the *FRT* flanked kanamycin resistance marker).--

Replace paragraph No. 262 on page 15 with the following paragraph, wherein additions are marked with underlining and underlined text is marked with double underline:

--The linear DNA fragment (1489 bp) containing a kanamycin selectable marker flanked by site-specific recombinase target sequences (*FRT*) was synthesized by PCR from plasmid pKD4 (Datsenko and Wanner, *supra*) with primer pairs,
T1(dxs) (5'-
TGGAAGCGCTAGCGGACTACATCATCCAGCGTAATAAATAACGTCTTGAGCGATT
GTGTAG-3') (SEQ ID NO:1) which contains a h1 homology arm (underlined, 41bp) chosen to match sequences in the upstream region of the *ispA* stop codon and a priming sequence (20 bp) and B1(T5) (5'-
CTCGAGGTGAAGACGAAAGGGCCTCGTGATACGCCTATTTTTATA
GGTTACATATGAATATCCT CCTTAG -3') (SEQ ID NO:2) that contains a h2 homology arm (underlined, 50 bp) chosen to match sequences in the 5'-end region of the promoter DNA fragment and a priming sequence (20 bp) (Figure 2). A second linear DNA fragment (154 bp) containing a phage promoter (P_{T5}) comprised of the –10 and –35 consensus sequences, lac operator (*lacO*), and ribosomal binding site (*rbs*) was synthesized by PCR from plasmid pQE30 (~~Qiagen~~ QIAGEN, Valencia, CA) with primer pairs, T2(T5) (5'-
TAACCTATAAAAA TAGGCGTATCACGAGG CCC-3') (SEQ ID NO:3) that contains a priming sequence (32 bp) and B2(dxs) (5'-GGAGTCGACCAG

TGCCAGGGTCGGGTATTTGG CAATATCAAAAC
TCATAGTTAATTTCTCCTCTTTAATG-3') (SEQ ID NO:4) that contains a h3 homology arm (underlined, 48bp) chosen to match sequences in the downstream region of the *dxs* start codon and a priming sequence (22 bp) (Figure 2). The underlined sequences illustrate each respective homology arm, while the remainder is the priming sequences for hybridization to complementary nucleotide sequences on the template DNA for the PCR reaction. The two resultant PCR fragments were the kanamycin selectable marker containing the homology arms (h1 and h2) and the P_{T5} promoter containing the homology arm (h3) as illustrated in Figure 2. Standard PCR conditions were used to amplify the linear DNA fragments with ~~AmpliTaq Gold®~~ AMPLI TAQ GOLD polymerase (Applied Biosystems, Foster City, CA) as follows; --

Replace paragraph No. 263 on page 16 with the following paragraph:

--After completing the PCR reactions, PCR products were purified using the ~~QIAquick~~ QIAQUICK Gel Extraction Kit™ (Cat. # 28704, QIAGEN Inc. Valencia, CA). Briefly, 50 µL of each PCR reaction mixture was run on a 1 % agarose gel. The band containing the amplified DNA fragment was excised from the agarose gel with a clean, sharp scalpel. The gel slice was weighted in a colorless plastic tube. After adding 3 volumes of buffer QG to 1 volume of gel, the tube was incubated at 50 °C for 10 min (or until the gel slice has completely dissolved). After the gel slice has dissolved completely, one gel volume of isopropanol was added to the sample and mixed by inverting the tube several times. In order to bind DNA, the sample was applied to the ~~QIAquick~~ QIAQUICK gel extraction column and centrifuged for 1 min. 750 µL of buffer PE was applied to the column for washing. After centrifuging for 1 min to remove the wash buffer, PCR DNA products were eluted with 10 µL of distilled water (dH_2O) by standing sample for 1 min, and then by centrifuging for 1 min. DNA Clean & Concentrator™ (Zymo Research, Orange, CA) was used to further purify the PCR DNA samples. After adding 2 volumes of DNA Binding Buffer to each volume of DNA sample, the samples were loaded into a Zymo-Spin Column (Zymo Research) and centrifuged at full speed (>10,000 g) for 5-10 sec. The PCR DNA sample retained in the column was washed twice with 200 µL of Wash Buffer by centrifuging at top speed for 5-10 seconds. The DNA was eluted with 6-8 µL of distilled water by spinning at top speed two times. The concentration of PCR DNA sample was about 0.5-1.0 µg/µL.--

Replace paragraph No. 266 on page 16 with the following paragraph:

--The electro-competent cells of *E. coli* MC1061 strain carrying pKD46 and pPCB15 were prepared as follows. *E. coli* MC1061 cells carrying pKD46 and pPCB15 were grown in SOB medium with 100 µg/mL ampicillin, 25 µg/ml chloramphenicol, and 1 mM L-arabinose at 30 °C to an OD_{600} of 0.5, following by chilling on ice for 20 min. Bacterial cells were centrifuged at 4,500 rpm using a ~~Sorvall®~~ SORVALL RT7 PLUS (Kendro Laboratory Products, Newton, CT) for 10 min at 4 °C. After decanting the supernatant, the pellet was

resuspended in ice-cold water and centrifuged again. This was repeated twice and the cell pellet was resuspended in 1/100 volume of ice-cold 10 % glycerol. --